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DATE: Monday, June 28, 2004

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☐	L1	serother$ or sero-therapy or sero-ther$	74
☐	L2	L1 and ($anthrax or $anthracis)	0
☐	L3	L1 and (anthrax$ or anthracis$)	0
☐	L4	L1 and (bacillu$)	7

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DATE: Monday, June 28, 2004

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☐	L2	L1 and ($anthrax or $anthracis)	0
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☐	L5	L1 and (bacillu$)	7
☐	L6	serother$ or sero-therapy or sero-ther$	142
☐	L7	L6 not l1	68
☐	L8	L7 and (anthrax$ or anthracis$)	1

END OF SEARCH HISTORY

plasma (plaz′mă)

1. The proteinaceous fluid (noncellular) portion of the circulating blood, as distinguished from the serum obtained after coagulation. Syn: blood plasma
2. The fluid portion of the lymph.
3. The fluid in which the fat droplets of milk are suspended.
4. A "fourth state of matter" in which, owing to elevated temperature, atoms have broken down to form free electrons and more or less stripped nuclei; produced in the laboratory in connection with hydrogen fusion (thermonuclear) research.

Syn: plasm [G. something formed]

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L9: Entry 5 of 36 File: PGPB Mar 25, 2004

DOCUMENT-IDENTIFIER: US 20040058403 A1
TITLE: Combinatorial protein library screening by periplasmic expression

Brief Description of Drawings Paragraph:
[0030] FIG. 8: Analysis of anti-PA antibody fragments selected using APEx (A) Signal Signal Plasmon Resonance (SPR) analysis of anti-PA scAb binding to PA. (B) Table of affinity data acquired by SPR. (C) FC Histogram of anti-PA scFv in pAPEx1 expressed in E. coli and labeled with 200 nM PA-Bodipy.TM. conjugate as compared with anti-methamphetamine (Meth) scFv negative control.

Brief Description of Drawings Paragraph:
[0031] FIG. 9: N-Terminal vs. C-Terminal anchoring strategy comparison. (A) Anti-digoxigenin Dig scfv, anti-PA M18 scFv and anti-methamphetamine Meth scFv expressed as N-terminal fusions in the pAPEx1 vector in E. coli specifically label with 200 nM of their respective antigen. (B) C-terminal fusions of same scFv in pAK200 vector specifically labeled with 200 nM of their respective antigen.

Detail Description Paragraph:
[0161] To demonstrate the ability of scFvs displayed via anchored periplasmic expression to specifically bind to large antigen conjugated fluorophores, E. coli were induced and labeled as described below expressing, via anchored periplasmic expression, an anti-protective antigen (PA) scFv (PA is one component of the anthrax toxin: a 83 kDa protein) or an anti-digoxigenin scFv. Histogram data of 10,000 events demonstrated specific binding to a PA-Cy5 antigen conjugated flourophore as compared to the cells expressing the an anti-digoxigenin scFv (FIG. 3A). To further illustrate this point, digoxigenin was coupled to phycoerythrin (PE), a 240 kDa fluorescent protein. Cells were labeled with this conjugate as described below. It was found that E. coli (10,000 events) expressing the anti-digoxigenin scFv via anchored periplasmic expression were labeled with the large PE-digoxigenin conjugate while those expressing a non-specific scFv via anchored periplasmic expression show little fluorescence (FIG. 3B).

Detail Description Paragraph:
[0231] A library of 1.times.10.sup.7 members was constructed by error-prone PCR of the gene for the anti-PA 14B7 scFv and was fused to the NlpA membrane anchoring sequence. DNA sequencing of 12 library clones selected at random revealed an average of 2% nucleotide substitutions per gene. Following induction of NIpA-[14B7 mutant scFv] synthesis with IPTG, the cells were treated with Tris-EDTA-lysozyme, washed, and labeled with 200 nM PA-BODIPY.TM.. Inner membrane integrity was monitored by staining with propidium iodide (PI). A total of 2.times.10.sup.8 bacteria were sorted using an ultra-high throughput Cytomation Inc. MoFlo droplet deflection flow cytometer selectively gating for low PI fluorescence (630 nm emission) and high BODIPY.TM. fluorescence. Approximately 5% of the cells sorted with the highest 530 nm fluorescence (FL1) were collected, immediately restained with PI alone and resorted as above. Since no antigen was added during this second sorting cycle, only cells expressing antibodies that have slow dissociation kinetics remain fluorescent. The plating efficiency of this population was low, presumably due to a combination of potential scFv toxicity (Somerville et al., 1994; Hayhurst and Harris, 1999), Tris-EDTA-lysozyme treatment and exposure to the high shear flow cytometry environment. Therefore, to avoid loss of potentially high

affinity clones, DNA encoding scFvs was rescued by PCR.TM. amplification of the approximately 1.times.10.sup.4 fluorescent events recovered by sorting. It should be be noted that the conditions used for PCR.TM. amplification result in the quantitative release of cellular DNA from the cells which have partially hydrolyzed cell walls due to the Tris-EDTA-lysozyme treatment during labeling. Following 30 rounds of PCR.TM. amplification, the DNA was ligated into pAPEx1 and transformed into fresh E. coli. A second round of sorting was performed exactly as above, except except that in this case only the most fluorescent 2% of the population was collected and then immediately resorted to yield approximately 5,000 fluorescent events.

Detail Description Paragraph:
[0237] Numerous antibody fragments to important therapeutic and diagnostic targets have been isolated from repertoire libraries screened by phage display. It is desirable to develop a means for rapid antigen binding analysis and affinity maturation of such antibodies without the need for time consuming subcloning steps. Antibodies are most commonly displayed on filamentous phage via fusion to the N-terminus of the phage gene 3 minor coat protein (g3p) (Barbas et al., 1991). During phage morphogenesis, g3p becomes transiently attached to the inner membrane via its extreme C-terminus, before it can be incorporated onto the growing virion (Boeke and Model, 1982). The antibody fragments are thus both anchored and displayed in the periplasmic compartment. Therefore, the inventors evaluated whether g3p fusion proteins can be exploited for antibody library screening purposes using the APEx format. The high affinity anti-PA M18 scFv discussed above, the anti-digoxin/digoxigenin 26-10 scFv, and an anti-methamphetamine scFv (Meth) were cloned in frame to the N-terminus of g3p downstream from a lac promoter in phagemid pAK200, which is widely used for phage display purposes and utilizes a short variant of gene III for g3p display (Krebber et al., 1997). Following induction with IPTG, cells expressing scFv-g3p fusions were permeabilized by Tris-EDTA-lysozyme and labeled with the respective fluorescent antigens (FIG. 9). High fluorescence was obtained for all three scFvs only when incubated with their respective antigens. Significantly, the mean fluorescence intensity of the scFvs fused to the N-terminus of g3p was comparable to that obtained by fusion to the C-terminus of the NlpA anchor. The results in FIG. 9 demonstrate that: (i) large soluble domains can be tethered N-terminally to a membrane anchor; (ii) antibody fragments cloned into phagemids for display on filamentous phage can be readily analyzed by flow cytometry using the APEx format, and (iii) scFv antibodies can be anchored on the cytoplasmic membrane either as N- or C-terminal fusions without loss of antigen binding.

Detail Description Paragraph:
[0242] An important issue with any library screening technology is the ability to express isolated clones at a high level. Existing display formats involve fusion to large anchoring sequences which can influence the expression characteristics of the displayed proteins. For this reason, scFvs that display well may not necessarily be amenable to high expression in soluble form as non-fusion proteins (Hayhurst et al., 2003). In contrast, the short (6 amino acid) tail that may be used for N-terminal tethering of proteins onto the cytoplasmic membrane in the current invention is unlikely to affect the expression characteristics of the fusion. Consistent with this hypothesis, all three affinity enhanced clones to the anthrax PA toxin isolated by APEx exhibited excellent soluble expression characteristics despite having numerous amino acid substitutions. Similarly, well-expressing clones have been obtained in the affinity maturation of a methamphetamine antibody, suggesting that the isolation of clones that can readily be produced in soluble form in bacteria at a large scale might be an intrinsic feature of selections with the invention.

Detail Description Paragraph:
[0243] In this example, the inventors employed APEx for affinity maturation purposes and have engineered scFvs to the B. anthracis protective antigen

exhibiting K.sub.D values as low as 21 pM. The scFv binding site exhibiting the highest affinity for PA has been humanized, converted to full length IgG and its neutralizing potential to anthrax intoxication is being evaluated in preclinical studies. In addition to affinity maturation, APEx can be exploited for several other protein engineering applications including the analysis of membrane protein topology, whereby a scFv antibody anchored in a periplasmic loop is able to bind fluorescent antigen and serves as a fluorescent reporter, and also, the selection of enzyme variants with enhanced function. Notably, APEx can be readily adapted to enzyme library sorting, as the cell envelope provides sites for retention of enzymatic catalytic products, thereby enabling selection based directly on catalytic turnover (Olsen et al., 2000). The inventors are also evaluating the utilization of APEx for the screening of ligands to membrane proteins. In conclusion, it has been demonstrated that anchored periplasmic expression has the potential to facilitate combinatorial library screening and other protein engineering applications.

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L9: Entry 9 of 36 File: PGPB Jan 22, 2004

DOCUMENT-IDENTIFIER: US 20040014707 A1
TITLE: Method for inhibiting the toxicity of Bacillus anthracis

Abstract Paragraph:
The cloning, expression and purification of a 32 kDa B. anthracis PA fragment (PA32) is described. This fragment has been expressed as a fusion construct to stabilized green fluorescent protein (EGFP-PA32). Both proteins bind to specific cell surface receptors. To confirm binding specificity, non-fluorescent PA83 or PA32 was used to competitively inhibit fluorescent EGFP-PA32 binding to cell receptors. The high intracellular expression levels and ease of purification make this recombinant protein an attractive vaccine candidate or therapeutic treatment for anthrax poisoning. Antibody fragments were isolated from a naive single-chain F.sub.v (scF.sub.v) library biopanned against PA83. Four scF.sub.v proteins were found to bind to PA83, the best one exhibiting a 10 nM K.sub.d. Two scF.sub.v proteins, scF.sub.v #1 and scF.sub.v #4, had similar affinities for PA32 and PA83, confirming the recombinant fragment was folded correctly.

Summary of Invention Paragraph:
[0003] The present invention relates generally to inhibiting anthrax toxicity and, more particularly, to the identification of human antibodies which bind to the protective antigen (PA83) of Bacillus anthracis, thereby disrupting cell receptor binding thereof.

Summary of Invention Paragraph:
[0004] Virulent Bacillus anthracis continues to represent a significant health threat, although the mechanism of anthrax intoxication is relatively well understood understood (See, e.g., "The Anthrax Toxin Complex" by S. H. Leppla, Sourcebook of Bacterial Protein Toxins, p. 277, J. E. Alouf (ed.), Academic Press, London (1991)). An 83 kDa form of protective antigen (PA83) is secreted from rapidly growing B. anthracis cells and binds to specific, but as yet unidentified, host cell surface receptors (See, e.g., "Anthrax protective antigen interacts with a specific receptor on the surface of CHO-K1 cells," by V. Escuyer and R. J. Collier, Infect. Immun. 59, 3381 (1991)). Subsequent cleavage by membrane-bound furin, and/or a furin-like protease, possibly PACE4, releases an amino terminal 20 kDa PA83 fragment resulting in receptor-bound PA63. The newly exposed surface on PA63 contains a single, high-affinity binding site that is recognized by the amino-termini of both the lethal factor (LF) and edema factor (EF) components of the toxin complexes. Endocytosis of the receptor/toxin complex into acidic endosomes elicits a conformational change in PA63, whereby the A subunits (LF or EF) of the toxin are released into the endosome. The PA63/receptor complexes then oligomerize into a heptameric ring. Lysosomal acidification and subsequent receptor release facilitates irreversible membrane insertion of the oligomeric PA63 pore. The pore permits transport of LF and/or EF into the cytoplasm where they elicit their respective toxicities. EF is a calcium/calmodulin-dependent adenylate cyclase that is toxic to most cell types and causes local inflammation and edema, but is not usually lethal. LF is a cell-type specific metalloprotease that cleaves MAP-kinase-kinases and several peptide hormones. Lethal factor is the major virulence factor associated with anthrax toxicity and is responsible for systemic shock and death associated with a hyper-oxidative burst and cytokine release from macrophages. Neither of the toxin A subunits are pathogenic in the absence of cytoplasmic

delivery by PA or mechanical means (See, "Macrophages are sensitive to anthrax lethal toxin through an acid-dependent process" by A. M. Friedlander J. Biol. Chem. 261, 7123 (1986)).

Summary of Invention Paragraph:
[0005] The crystal structures of PA83 and heptameric PA63 have been solved (See, e.g., "Crystal-structure of the anthrax toxin protective antigen" by C. Petosa et al., Nature. 385, 833 (1997)). These structural data support the experimental data (See, e.g., "Characterization of lethal factor-binding and cell-receptor binding domains of protective antigen of Bacillus anthracis using monoclonal-antibodies" by S. F. Little et al., Microbiology-UK. 142, 707 (1996) and "The carboxyl-terminal end of protective antigen is required for receptor-binding and anthrax toxin activity" by Y. Singh et al., J. Biol. Chem. 266,15493 (1991)) that indicate that domain 4, the carboxy-terminus of PA63, is responsible for receptor-mediated uptake of the toxin complex.

Summary of Invention Paragraph:
[0008] The human anthrax vaccine used in the United States and other western countries consists of aluminum hydroxide-adsorbed supernatant material from cultures of toxigenic, non-encapsulated B. anthracis strains. Current protocols for isolating native PA83, the primary immunogen in the vaccine, from culture supernatants are time- and cost-intensive. Immunization with this vaccine can cause local edema and erythema, probably due to trace amounts of LF or EF, and frequent boosters are required. It has been shown that only immunization with PA, but not LF or EF, can protect against lethal B. anthracis challenge in a guinea pig model.

Summary of Invention Paragraph:
[0009] It has been suggested that reduced protection seen with some recombinant PA vaccine preparations may be due to lack of contaminating LF or EF. Y. Singh et al. in "A deleted variant of Bacillus anthracis protective antigen is non-toxic and blocks anthrax toxin action in vivo," J. Biol. Chem. 264, 19103 (1989) used recombinant PA molecules that bind receptors, but not LF or EF. Their approach was to mutate a PA83 protease site to prevent the EF/LF binding site from being exposed by furin cleavage and PA20 release. Immunization of guinea pigs with this cleavage-resistant PA vaccine led to significant protection against otherwise lethal anthrax infection (See, "Study of immunization against anthrax with the purified recombinant protective antigen of Bacillus anthracis" by Y. Singh, Infect. Immun. 66, 3447 (1998)).

Summary of Invention Paragraph:
[0012] Another object of the invention is to generate a recombinant PA fragment to compete with native PA83 for its receptors which can be purified such that no anthrax toxin components remain after the manufacturing process.

Summary of Invention Paragraph:
[0013] Still another object of the invention is to provide a method for rapid screening for inhibitors of anthrax toxicity.

Summary of Invention Paragraph:
[0014] Yet another object of the present invention is to identify antibodies against domain 4 of PA83 as candidates for anthrax toxicity neutralization by interfering with PA83 binding to its host receptors.

Detail Description Paragraph:
[0030] Briefly, the present invention includes the identification of human scF.sub.v as potential prophylactics or therapeutics against anthrax poisoning, and the use of recombinant PA32 as a potential prophylactic or therapeutic agent to compete with anthrax toxins for cellular receptors during active infection. A method method for identifying antibodies that bind native B. anthracis protective antigen PA83 using a high-throughput flow cytometric competition assay has been developed.

This assay employs a fluorescently tagged form of PA32, and a naive, human single-chain F.sub.v (scF.sub.v) phagemid library has been investigated thereby. Certain scF.sub.v that bind to PA83 have been isolated by the present inventors using biopanning. The recombinant PA32 retains its ability to undergo receptor-mediated uptake and is also recognized by the scF.sub.v isolated by biopanning which recognized PA83. At least one scFv antibody fragment that blocks binding of the fluorescently tagged PA32 moiety to cell surface receptors has been identified. Selected scF.sub.v were first isolated from the naive library by biopanning against PA83. Soluble, monomeric scFv were then characterized for affinity and screened for their capacity to disrupt receptor mediated binding of PA. Four of the scFv were found to bind to PA83, as determined by surface plasmon resonance, the tightest binder exhibiting a K.sub.d of 50 nM. Two of these scFv displayed similar affinities affinities for both natural PA83 and the 32 kDa carboxy-terminal PA fragment (PA32). (PA32). Fusion of EGFP to PA32 facilitated development of the flow-cytometric assay of the present invention that showed that one of the scFv actually disrupts PA receptor binding. The present method can now be used as a rapid assay for other small molecule inhibitors of PA binding to cell receptors.

Detail Description Paragraph:
[0039] D. PA83 isolation: PA83 was purified as described in "Purification of anthrax-toxin components by high-performance anion-exchange; gel-filtration and hydrophobic-interaction chromatography" by C. P. Quinn et al., J. Biochem. 252, 753 (1988). Briefly, clarified supernatant was collected from a 20 L culture of pX02 cured Steme strain B. anthracis containing mutant LF and EF. A 20% ammonium sulfate precipitation was used to enrich PA83 relative to other secreted proteins. Subsequent FPLC purifications were performed using Mono-Q and gel filtration (SEPHADEX G-75) columns. The final protein preparation was >90% pure as determined by SDS-polyacrylamide gel electrophoresis.

Detail Description Paragraph:
[0046] A. SDS-PAGE analysis of purified recombinant and natural PA proteins: Purification of the native PA83 as described hereinabove yielded a >90% pure protein preparation. Purification of recombinant anthrax proteins was performed by immobilized metal affinity chromatography (IMAC) in a single step. All IMAC purified proteins were >95% homogeneous after elution as determined by SDS-polyacrylamide gel electrophoresis. A recombinant PA comprised of the carboxy-terminal 32 kDa is highly soluble in E. coli and did not appear to be toxic to the bacteria. PA32 was also cloned as a fusion protein with a green fluorescent protein variant (EGFP) attached to its amino terminus. The EGFP-PA32 fusion was designed for use in a flow cytometry assay where inhibitors of PA receptor binding could be analyzed. As controls for the different assays, His-tagged EGFP (full length 31 kDa), and chimeric EGFP-EF32 were expressed and purified similar to the recombinant PA32 proteins.

Detail Description Paragraph:
[0050] Preliminary work by S. H. Leppla in "Production and purification of anthrax toxin," Meth. Enz. 165,103 (1988) to generate a recombinant carboxyl-terminal PA fragment indicated a fragment from T624-G735 could not compete with radio-labeled PA83 for receptors. This work was completed prior to the crystal structure solution. It is believed by the present inventors that the reason PA32 (domains 3 and 4) is able to compete for receptors is that the protein is structurally more stable than the T624-G735 fragment tested by Leppla and co-workers. This hypothesis is supported by the present SPR results that show several of the anti-PA83 scF.sub.v also bind to PA32; that is, the molecule evidently is folded in a manner that preserves epitopes common to native PA. The ability of PA32 to interact with its host cell surface receptor (FIG. 2) and be internalized favorably supports the possibility that this PA fragment may be effective as an anti-toxin treatment during anthrax infection.

Detail Description Paragraph:

[0052] B. Inhibition of receptor-mediated EGFP-PA32 binding by scFv targeted to PA: This flow cytometric analysis was subsequently used to screen scFv for their ability ability to disrupt PA-receptor interactions. Incubation of scFv4 with EGFP-PA32 at a a 1:1 molar ratio was able to significantly (>80%) abolish receptor-mediated binding binding of EGFP-PA32 to A549 cells (FIG. 4). A ten fold molar excess of scFv4 showed showed little additional inhibition (data not shown) as would be expected for a monovalent competition. The scF.sub.v#1, which can recognize EGFP-PA32 (FIG. 1), showed minimal inhibition of EGFP-PA32 binding by this assay. This indicates that it it does not recognize or mask an essential structure necessary for receptor recognition. The scF.sub.v#12 did not inhibit binding as expected since it did not recognize the C-terminal PA32 protein (FIG. 1). For comparison, the means of the dose-dependent competition with unlabeled PA are also shown in FIG. 4. These data indicate the flow cytometric assay is a sensitive and specific method to identify molecules which inhibit receptor-mediated anthrax toxin binding, and that one of the the scFv selected has the potential to inhibit PA binding to cells in a therapeutically useful fashion.

Detail Description Paragraph:
[0054] The current human, Bacillus anthracis vaccine used in the United States is an aluminum hydroxide adjuvant conjugated to natural PA83 secreted from a virulent bacteria. Protective effects of this compound, as well as a recombinant, non-toxic PA83 have been tested (See, e.g., B. E. Ivins, et al., Infection and Immunity 60, 662 (1992) and Y. Singh et al., Infection and Immunity 66, 3447 (1998). These vaccines were tested in guinea pigs, the standard model for human anthrax, and it was found that the recombinant PA83, but not the aluminum hydroxide adjuvant/PA83, could protect animals from lethal anthrax infection. This data supports testing of new anthrax vaccines and suggests recombinant PA32 alone or conjugated to novel adjuvants, such as monophosphoryl lipid A (MPL), might be more effective than the current licensed human vaccine.

Detail Description Paragraph:
[0055] Anti-PA83 polyclonal antisera from guinea pigs was able to protect non-immunized animals from lethal anthrax challenge (See S. F. Little et al. Infection and Immunity 65, 5171 (1997). Monoclonal antibodies were able to delay time of death but were not protective. This data suggests a combination of the scF.sub.v of the present invention might be effective in protection or treatment of anthrax infection in humans. Delivery of either PA32 or scF.sub.v could be accomplished in two ways: (1) as DNA vaccines such that the host cells (e.g., human epithelial cells) would express the proteins after a delay period; or (2) as purified proteinacious components which are instantly available for therapeutics. DNA immunization is proving very effective in generating host immunity when an immunogen's (e.g., PA32) DNA sequence is injected (See e.g., P. Young, ASM News 63, 659 (1997) and D. M. Klinman et al., J. Immunol 160, 2388 (1998)). Additionally, systems are being developed in which functional scFv can be expressed from DNA vectors in mammallian cells (See, L. Persic et al., Gene 187, 9 (1997)). The uses of purified proteins to compete with PA83 binding (i.e., PA32) or inhibit binding (i.e., scF.sub.v) have not been tested.

Detail Description Table CWU:
4TABLE Analysis of anti-PA83 selected single-chain F.sub.v. ELISA.sup.a Mono/Di/Trimer.sup.b K.sub.d (M).sup.c ScF.sub.v#1 0.75 8:2:0 1.9 .times. 10.sup.-7 SEQ. ID No. 9 ScF.sub.v#4 0.20 9:1:0 3.1 .times. 10.sup.-7 SEQ. ID No. 10 ScF.sub.v#5 1.14 1:2:7 ND.sup.d ScF.sub.v#12 0.27 7:3:0 1.1 .times. 10.sup.-6 SEQ. ID No. 11 ScF.sub.v#24 0.30 9:1:0 4.3 .times. 10.sup.-7 SEQ. ID No. 12 .sup.aWells of 96 well plate coated with 1 .mu.g PA83. Anti-M13-HRP conjugated antibody was used to generate signal. .sup.bMultimer content was determined by SEPHADEX-75 separation of IMAC purified protein as described hereinabove. .sup.cAffinity was determined using 4 concentrations of each scF.sub.v by BIACORE. .sup.dND, not done.

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L11: Entry 12 of 138 File: PGPB Jan 22, 2004

DOCUMENT-IDENTIFIER: US 20040014707 A1
TITLE: Method for inhibiting the toxicity of Bacillus anthracis

CLAIMS:

1. A method for inhibiting the toxicity of Bacillus anthracis, which comprises the steps of introducing at least one type of DNA-encoding single-chain F.sub.v fragment (scF.sub.v) protein into host mammalian cells, whereby the host mammalian cells produce an immunoglobulin that functions in an immune response against protective antigen PA83 of Bacillus anthracis.

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L14: Entry 7 of 64 File: PGPB Feb 5, 2004

DOCUMENT-IDENTIFIER: US 20040024343 A1
TITLE: Methods and devices for treating severe peripheral bacterial infections

Detail Description Paragraph:
[0031] Inside of the column is the bacterium and toxin binding means and the associated solid support. At the inlet and outlet ends are 80 micron nylon nets (18) for retaining the solid support within the container while allowing blood cells to pass through safely. The solid support comprises agarose particles (20), such as CN-Br activated Sepharose 6B available from Amersham Biosciences (Piscataway, N.J.). Antibacterial antibodies and anti-bacterial toxin antibodies (22) are affixed to the agarose support by conventional means according to instructions from the manufacturer using sterile solution and glassware that has been previously sterilized. For example, in the case of an EBTR unit for severe anthrax infection, once can use affinity-purified goat anti bacillus anthracis antibodies and goat anti bacillus anthracis toxin antibodies available from Scantibodies Laboratory, Inc. (Santee, Calif.).

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L14: Entry 40 of 64 File: PGPB Apr 4, 2002

DOCUMENT-IDENTIFIER: US 20020039588 A1
TITLE: Compounds and methods for the treatment and prevention of bacterial infection infection

Detail Description Paragraph:
[0055] The multiple mutants of anthrax PA were constructed, expressed, purified, and assayed to determine whether they have reduced activity compared to wild-type PA. In particular, these mutants were assayed for the ability to bind PA ligands and receptors; to form prepores, SDS-resistant oligomers, and pores; and to translocate ligands across membranes. Based on the x-ray structure of PA, the mutated residues are predicted to project into the lumen of the PA prepore. PA mutants, or fragments thereof, with reduced or no detectable ability to form pores in membranes can be used as vaccines for the induction of protective antibodies to prevent anthrax infection. In addition, these mutants might be more effective than wild-type PA in treating anthrax infection because of their reduced ability to translocate EF and LF secreted by Bacillus anthracis in the infected mammal.

Detail Description Paragraph:
[0056] These point mutants and the previously reported deletion mutant lacking residues 302-325 of putative membrane spanning loop 2 of domain 2 (.DELTA.D2L2) (Miller et al., Biochemistry 38:10432-10441, 1999) were further characterized to determine whether they could act as dominant negative inhibitors by reducing the pore formation of wild-type PA. This inhibition could result from the binding of ligands or receptors by the mutants so that fewer molecules were available for wild type PA to bind. The mutants could also form oligomers with wild-type PA that have reduced or no detectable ability to form pores and translocate ligands. Dominant negative PA mutants, and fragments thereof, could be used as vaccines to elicit protective antibodies for the prevention or treatment of anthrax infection, as described above. Additionally, mutants or fragments with dominant negative activity could be used as therapeutics to treat anthrax infection by inhibiting the activity of PA secreted by Bacillus anthracis in the infected mammal. Because dominant negative mutants can induce the production of protective antibodies and inhibit the activity of PA produced by the infecting bacteria, they can be used as a combination vaccine/therapeutic that is particularly effective in treating individuals suffering from, or at risk of developing, anthrax infection. Besides the need to abrogate toxin action as quickly as possible, it is also important to vaccinate individuals who have been exposed to aerosolized B. anthracis spores. This vaccination is essential to guard against delayed contraction of anthrax by germination of spores that can remain in the body for prolonged periods (at least a month).

Detail Description Paragraph:
[0063] Dominant negative PA may also be useful as a basis for a new vaccine against anthrax. As its name connotes, PA induces protective antibodies against anthrax, and indeed is the major immunogen of the vaccine currently licensed in the United States. The .DELTA.D2L2, K397D+D425K, and F427A mutants described herein exhibit little or no diminution in immunogenicity relative to wild-type PA in Fisher rats. We have also found mutants that are unexpectedly dominant negative, such that administration of a 0.25:1 ratio of mutant to wild-type PA did not result in any detectable symptoms of anthrax infection in a rat model. Purified wild-type PA is

under consideration as a replacement for the currently licensed vaccine, and if a dominant negative form of PA proves efficacious therapeutically, it might fulfill this role as well, eliminating the need to develop two almost identical pharmaceuticals.

Detail Description Paragraph:
[0113] Anti-PA antibodies may be used to measure PA protein in a biological sample such as serum, by contacting the sample with the antibody and then measuring immune complexes as a measure of the PA protein in the sample. Thus, these antibodies may be used in kits to determine whether a subject has been exposed to anthrax toxin.

Detail Description Paragraph:
[0114] Antibodies to PA can also be used as therapeutics for the treatment or prevention of anthrax infection. If a anti-PA antibody that binds wild-type PA but does not bind a dominant negative PA mutant is administered to a subject for passive immunization against anthrax infection, a dominant negative PA mutant may also be administered to the same subject as a therapeutic to inhibit the activity of wild-type PA. Because the administered anti-PA antibody does not react with the therapeutic dominant negative PA mutant, the anti-PA antibody should not reduce the ability of the dominant negative PA mutant to inhibit wild-type PA. Additionally, an anti-PA antibody that does not react with a therapeutic dominant negative PA mutant may be used to determine the amount of wild-type PA present in a sample from a subject who has been treated with the dominant negative PA mutant.

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L14: Entry 54 of 64 File: USPT Dec 11, 2001

DOCUMENT-IDENTIFIER: US 6329156 B1
TITLE: Method for screening inhibitors of the toxicity of Bacillus anthracis

Government Interest Text (2):
The present invention relates generally to inhibiting anthrax toxicity and, more particularly, to the identification of human antibodies which bind to the protective antigen (PA83) of Bacillus anthracis, thereby disrupting cell receptor binding thereof. This invention was made with government support under Contract No. W-7405-ENG-363 awarded by the U.S. Department of Energy to The Regents of The University of California. The government has certain rights in the invention.

Brief Summary Text (12):
Yet another object of the present invention is to identify antibodies against domain 4 of PA83 as candidates for anthrax toxicity neutralization by interfering with PA83 binding to its host receptors.

Detailed Description Text (47):
This flow cytometric analysis was subsequently used to screen scFv for their ability to disrupt PA-receptor interactions. Incubation of scFv4 with EGFP-PA32 at a 1:1 molar ratio was able to significantly (>80%) abolish receptor-mediated binding of EGFP-PA32 to A549 cells (FIG. 4). A ten fold molar excess of scFv4 showed little additional inhibition (data not shown) as would be expected for a monovalent competition. The scFv1, which can recognize EGFP-PA32 (FIG. 1), showed minimal inhibition of EGFP-PA32 binding by this assay. This indicates that it does not recognize or mask an essential structure necessary for receptor recognition. The scF.sub.v #12 did not inhibit binding as expected since it did not recognize the C-terminal PA32 protein (FIG. 1). For comparison, the means of the dose-dependent competition with unlabeled PA are also shown in FIG. 4. These data indicate the flow cytometric assay is a sensitive and specific method to identify molecules which inhibit receptor-mediated anthrax toxin binding, and that one of the scFv selected has the potential to inhibit PA binding to cells in a therapeutically useful fashion.

Detailed Description Text (50):
Anti-PA83 polyclonal antisera from guinea pigs was able to protect non-immunized animals from lethal anthrax challenge (See S. F. Little et al. Infection and Immunity 65, 5171 (1997). Monoclonal antibodies were able to delay time of death but were not protective. This data suggests a combination of the scF.sub.v of the present invention might be effective in protection or treatment of anthrax infection in humans. Delivery of either PA32 or scF.sub.v could be accomplished in two ways: (1) as DNA vaccines such that the host cells (e.g., human epithelial cells) would express the proteins after a delay period; or (2) as purified proteinacious components which are instantly available for therapeutics. DNA immunization is proving very effective in generating host immunity when an immunogen's (e.g., PA32) DNA sequence is injected (See e.g., P. Young, ASM News 63, 659 (1997) and D. M. Klinman et al., J. Immunol 160, 2388 (1998)). Additionally, systems are being developed in which functional scFv can be expressed from DNA vectors in mammallian cells (See, L. Persic et al., Genes 187, 9 (1997)). The uses of purified proteins to compete with PA83 binding (i.e., PA32) or inhibit binding (i.e., scF.sub.v) have not been tested.

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L2: Entry 7 of 13 File: PGPB May 8, 2003

DOCUMENT-IDENTIFIER: US 20030088074 A1
TITLE: Recombinant bivalent monospecific immunoglobulin having at least two variable variable fragments of heavy chains of an immunoglobulin devoid of light chains

Abstract Paragraph:
The present invention relates to fragments, especially variable fragments of immunoglobulins which are by nature devoid of light chains, these fragments being nevertheless capable of exhibiting a recognition and binding activity toward specific antigens. The present invention further relates to the use of such immunoglobulin fragments formed of at least one heavy chain variable fragment or derived therefrom, for therapeutic or veterinary purposes and especially for passive immunotherapy or serotherapy.

CLAIMS:

7. Variable fragment of a heavy chain of an immuglobulin encoded by a nucleotide sequence according to claim 5 wherein the antigen is a bacterial toxin or toxoid chosen among those of the following bacteria: Clostridium, especially Clostridium Botulinum or Clostridium Perfringens, Staphylococcus, Pseudomonas, Pasteurella, Yersinia, Bacillus Anthracis, Neisseria, Vibrio, especially Vibrio cholera, enterotoxic E. Coli, Salmonella, Shigella, Listeria.

25. Pharmaceutical composition according to claim 16 for the treatment by passive immunisation, of infection or acute intoxication by toxins such as those of Clostridium, especially Clostridium Botulinum or Clostridium Perfrinaens, Staphylococcus, Pseudomonas, Pasteurella, Yersinia, Bacillus AnthracisNeisseria, Vibrio, especially Vibrio cholera, enterotoxic E. Coli, Salmonella, Shigella, Listeria or anemonies, coral, jellyfish, spiders, beas, wasps, scorpions, snakes, including those belonging to the families of Viperidae, Crotalidae, Lapidea.

26. immunoglobulin variable fragment according to anyone of claims 2 to 6 or 19, or a construct according to anyone of claims 17, 18,20 to 23 for use for the treatment by passive immunisation, of infection or acute intoxication by toxins such as those of Clostridium, especially Clostridium Botulinum or Clostridium Perfrinaens, Staphylococcus, Pseudomonas, Pasteurella, Yersinia, Bacillus Anthracis, Neisseria, Vibrio, especially Vibrio cholera, enterotoxic E. Coli, Salmonella, Shigella, Listeria or anemonies, coral, jellyfish, spiders, beas, wasps, scorpions, snakes, including those belonging to the families of Viperidae, Crotalidae, Lapidea.

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L2: Entry 11 of 13 File: DWPI Mar 22, 2001

DERWENT-ACC-NO: 2001-244703
DERWENT-WEEK: 200253

TITLE: Acellular immunogenic composition against Bacillus anthracis, useful in human human or veterinary vaccines, comprises protective antigen and killed spores

Basic Abstract Text (1):
NOVELTY - An acellular immunogenic composition (A) for inducing an immune response against infection by Bacillus anthracis comprising a protective antigen (PA) and killed (optionally purified) spores of a mutant strain of B. anthracis, in at least one vehicle, is new.

Basic Abstract Text (2):
DETAILED DESCRIPTION - An acellular immunogenic composition (A) for inducing an immune response against infection by Bacillus anthracis comprising a protective antigen (PA) and killed (optionally purified) spores of a mutant strain of B. anthracis, in at least one vehicle, is new. The mutant has at least one mutation in at least one gene encoding for the toxic effects of the bacterium or lacks at least one of plasmids pOX1 and pOX2.

Basic Abstract Text (5):
(2) the strain of B. anthracis RPLC2, deposited as CNCM I-2270;

Basic Abstract Text (6):
(3) use of antibodies (Ab1) directed against the specified spores for passive immunization;

Basic Abstract Text (7):
(4) a purified antigenic preparation (B) derived from B. anthracis spores and containing at least one of the exoantigens of molecular weights 15, 30, 55 and over 200 kilo Dalton; and

Basic Abstract Text (12):
USE - (A), particularly when formulated as vaccines, are useful in human and veterinary medicine to protect against anthrax. They can also be used to raise antibodies against the mutant spores, for use in passive immunization, particularly in combination with antibiotic therapy.

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L1: Entry 4 of 10 File: DWPI Jan 20, 2001

DERWENT-ACC-NO: 2001-255358
DERWENT-WEEK: 200126

TITLE: Preparation of semiproduct of equine anti-anthrax globulin

INVENTOR: KOMISSAROV, A V; KOMOSKO, G V ; LESHCHENKO, A A ; LUB M YU, ; PIMENOV, E V ; VASILEV, P G ; ZHUCHIKHIN YU, S

PATENT-ASSIGNEE: MICROBIOLOGY RES INST DEFENCE MINISTRY (MICRR)

PRIORITY-DATA: 1999RU-0117037 (August 11, 1999)

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PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	RU 2161985 C1	January 20, 2001		000	A61K039/40

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
RU 2161985C1	August 11, 1999	1999RU-0117037	

INT-CL (IPC): A61 K 39/40

ABSTRACTED-PUB-NO: RU 2161985C
BASIC-ABSTRACT:

NOVELTY - Method of preparing the dried semiproduct of anti-anthrax globulin involves additional addition of polyclucin sucrose to a solution of immunoreactive gamma- and beta-globulin fractions isolated from the equine blood serum proteins in the ratio of components = 92.5 : 0.5 : 7.0 wt.%.

DETAILED DESCRIPTION - Method of preparing the dried semiproduct of anti-anthrax globulin involves additional addition of polyclucin sucrose to 8.5-9.5% solution of immunoreactive gamma- and beta-globulin fractions isolated from the equine blood serum proteins in the ratio of components = 92.5 : 0.5 : 7.0 wt.%. Invention provides the increase of storage period of globulin semiproduct before its processing to the ready preparation at simultaneous retention of its quality indices of the ready preparation determined by normative standards for equine anti-anthrax liquid globulin. Obtained substance is dried by the sublimation method. Invention can be used for stabilization of physical-chemical and immunobiological properties of anti-anthrax globulin semiproducts.

USE - Veterinary science, biotechnology, immunology.

ADVANTAGE - Increased period of storage of semiproduct.

ABSTRACTED-PUB-NO: RU 2161985C
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/0

DERWENT-CLASS: B04 C03
CPI-CODES: B04-B04D4; B04-N02; B07-A02; B14-A02; B14-S12; C04-B04D4; C04-N02; C07-A02; C14-A02; C14-S12;

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L11: Entry 111 of 138 File: DWPI May 15, 2003

DERWENT-ACC-NO: 2003-430672
DERWENT-WEEK: 200340

TITLE: New purified affinity-matured recombinant antibody with binding specificity for Bacillus anthracis protective antigen, useful for diagnosing, preventing or treating anthrax or other bacterial infections

INVENTOR: GEORGIOU, G; IVERSON, B L ; MAYNARD, J A

PATENT-ASSIGNEE: UNIV TEXAS SYSTEM (TEXA)

PRIORITY-DATA: 2001US-332849P (November 5, 2001)

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PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	WO 2003040384 A1	May 15, 2003	E	024	C12P021/08

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
WO2003040384A1	November 5, 2002	2002WO-US35567	

INT-CL (IPC): C07 K 16/00; C12 M 3/00; C12 P 21/08; G01 N 33/53

ABSTRACTED-PUB-NO: WO2003040384A
BASIC-ABSTRACT:

NOVELTY - A purified affinity-matured recombinant antibody or its portion having binding specificity for a proteinaceous toxin or a Bacillus anthracis protective antigen, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a process for producing antibodies to bacterial toxins that produce an immune response protective against and/or alleviates the symptoms of bacterial infection, comprising:

(a) culturing a microorganism that expresses a bacterial toxin or its fragments in a a bacteriophage that is expressed in the microorganism;

(b) constructing a bacteriophage library that expressed affinity matured antibodies or its portions;

(c) contacting the bacteriophage library with the microorganism; and

(d) selecting bacteriophage with at least three-fold higher affinity to the bacterial toxin;

(2) a method of treating a host having or at risk of infection by B. anthracis, comprising administering to a host a composition comprising an affinity matured antibody against a proteinaceous toxin or its portion derived from a monoclonal antibody;

(3) a pharmaceutical composition for the treatment of a pathogenic infection, comprising the above antibody or its portion in a carrier;

(4) a diagnostic device comprising an immobilized antibody or its portion cited above, or an immobilized proteinaceous toxin that has a binding affinity for the above antibody or its portion; and

(5) a method for diagnosing exposure to a proteinaceous toxin, comprising contacting the above diagnostic device with a fluid and analyzing the device for indication of a reaction between the proteinaceous antigen and the antibody.

ACTIVITY - Antibacterial.

Protection to toxin intoxication was examined in the Fisher 344 rat model. Rats were challenged with a 10 x MLD amount of protective antigen (PA) and lethal factor (LF) (0.160 and 0.064 mg/kg respectively) and survival is monitored for five hours. Control rats receiving phosphate buffered saline (PBS) only as treatment expired after 91 plus or minus 7 minutes. Animals receiving 14B7 scFv 5 minutes after toxin challenge exhibited a small but significant increase in time to death (110 plus or minus 17 minutes, p less than 0.05). Administration of the A2E scFv results in more dramatic delays in both the onset of symptoms and time to death (mean TTD (Time to Death) 237 plus or minus 63 minutes with two-survivors).

MECHANISM OF ACTION - Gene therapy; Vaccine.

USE - The composition and methods are useful in diagnosing, preventing or treating infections caused by B. anthracis and other bacterial toxins.

ABSTRACTED-PUB-NO: WO2003040384A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/7

DERWENT-CLASS: B04 D16 S03
CPI-CODES: B04-B04B1; B04-B04C1; B04-B04D; B04-B04G; B04-C01; B04-G0100E; B04-N03; B11-C07A; B11-C07B; B14-A01; B14-G01; B14-S03; B14-S11B; D05-H07; D05-H08; D05-H09; D05-H11; D05-H17A1;
EPI-CODES: S03-E14H4;

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L2: Entry 12 of 13 File: DWPI May 8, 2003

DERWENT-ACC-NO: 1996-478745
DERWENT-WEEK: 200337

TITLE: Immunoglobulin heavy chain variable fragments - encoded by Camelid nucleotide nucleotide sequences, useful for passive immunisation.

Basic Abstract Text (2):
USE - The VHH fragment can be used in a pharmaceutical compsn. for passive immunisation of humans or other animals against infection or acute intoxication by toxins such as those produced by microorganisms, such as Clostridium botulinum, C. perfingens, Staphylococcus, Pseudomonas, Pasteurella, Yersinia, Bacillus Anthracis, Neisseria, Vibrio cholera, enterotoxic E. coli, Salmonella, Shigella, and and Listeria (claimed). The pharmaceutical compsn. can also be for passive immunisation against a toxin produced by sea anemones, corals, jellyfish, spiders, bees, wasps, scorpions or snakes, inc. those belonging to the family Viperidae, Crotalidae, and Lapidea (claimed).